Bioefficacy of Extracts of *Hyptis suaveolens*, *Eugenia aromatica* and *Azadirachta indica* as Field Protectants of Pawpaw Against *Paracoccus marginatus* Will. and Granara de Willink in Ibadan, Southwest Nigeria

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Abstract

The papaya mealybug, *Paracoccus marginatus* is a small sized polyphagous invasive hemipteran bug that attacks several genera of host plants, causing considerable yield loss. Commonly used insecticidal control is being discouraged due to environmental and human health hazards. Information on the control of this pest with botanicals is scanty, therefore the control using selected botanicals was investigated. Aqueous and ethanolic extracts of three botanicals: *Hyptis suaveolens*, *Eugenia aromatica* and *Azadirachta indica* were evaluated in screen cages (1.80x0.60x1.50m) for contact toxicity following standard procedures. Cypermethrin® (100 ml a.i ha⁻¹) was used as a standard check. The secondary metabolites in the three plants were analyzed for saponins, alkaloids, terpenoids, phenols, flavonoids and cardiolides following standard procedures. Effective concentration was determined using probit analysis. Both aqueous and ethanolic extracts of the botanicals caused adult mortality of *P. marginatus*. However, the difference in percentage mortality in aqueous extracts of *A. indica* (54.28±1.7), *H. suaveolens* (47.5±4.1) and *E. aromatica* (48.6±1.1) were not significant (P>0.05). Percentage mortality of ethanolic extracts of *A. indica* (87.40±4.8) was significantly higher (P<0.05) than both *E. aromatica* (74.40.16±2.4) and *H. suaveolens* (72.10.58±2.8). Concentration of the secondary metabolites varied in the botanicals with *H. suaveolens* having higher flavonoids (107.08mg/100g), cardiolides (46.06 mg/100g) and terpenoids (3.17mg/100g) while *A. indica* had higher saponins (10.33mg/100g) and phenols (1938.48mg/100g) while *E. aromatica* had comparatively higher alkaloids (0.59%). The LC₅₀ ranged from 3.61mg/kg in *A. indica*, to 4.64mg/kg in *E. aromatica* and 4.52mg/kg in *H. suaveolens*. The ethanolic extracts of botanicals evaluated were more effective than the aqueous extracts and can serve as a substitute to the synthetic insecticides for the control of *P. marginatus*.

Keywords: *Paracoccus marginatus*, Botanicals, *Carica papaya*, Mealybug, Insecticide.

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INTRODUCTION

The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, is a small sized, polyphagous and highly invasive hemipteran pest that attacks several genera of host plants, including economically important tropical fruits, vegetables and ornamentals (Tanwar *et al.*, 2010). It is believed to be native to Mexico and/or Central America although it has never gained status as a serious pest there, probably due to the presence of an endemic natural enemy complex (Miller *et al.*, 1999). The papaya mealybug feeds on the sap of plants by inserting its stylets into the epidermis of the leaf, as well as into the fruit and stem. In doing so, it injects toxic substances into the leaves which cause chlorosis, plant stunting, leaf deformation, early leaf and fruit drop, a heavy build-up of honeydew and death of the plant. Symptoms start as bunchy top and end up in a candle stick appearance in young plants. Heavy infestations are capable of rendering fruits inedible due to the build-up of thick white wax (Walker *et al.*, 2011).

The mealybug if not controlled, can cause multi-million dollar loss to numerous agricultural products (Walker *et al.*, 2011). Since the first report of this mealybug in Africa in 2010, it has caused great concern in Ghana, where over 2000ha of papaya were under cultivation before it arrived. It was estimated that the pest destroyed over 80% of papaya farms, with a resulting yield loss of over 60% and a drastic reduction of the papaya orchards to less than 400%. As a result, export earnings for the papaya industry significantly reduced and about 1700 persons in the sector lost their jobs (Georgen *et al.*, 2011). The economic and ecological impacts of an imminent outbreak in Nigeria where, according to FAO (2010) Over 90% of papaya in West Africa is grown, are likely to be even more dramatic (Georgen

et al., 2011). There are however reports that the pest has been a problem of papaya since 2012 in Ibadan, South-western Nigeria (Aduramigba, *pers comm.*). The use of synthetic insecticides such as organo-phosphates (malathion, chlorpyrifos and diazinone) or carbamates (dimethoate, carbaryl and acephate) to control *P. marginatus*, (Walker *et al.*, 2011) is being deemphasized owing to the danger of possible outbreak of secondary pests normally kept in check by natural enemies (Omoloye, 2008; Prishanthini and Vinobaba, 2014). Due to the other problems such as human health hazards, undesirable side effects and environmental pollution caused by the continuous use of synthetic chemical pesticides, there is renewed interest in the application of botanical pesticides for crop protection (Prishanthini and Vinobaba, 2014).

Botanical pesticides are safe and biodegradable (Devlin and Zettel, 1999) and their use in crop protection is a practical sustainable alternative. They maintain biological diversity of predators and reduce environmental contamination and human health hazards (Grange and Ahmed, 1988).

Information on the control of *P. marginatus* with botanicals is scanty; hence the experiments reported here to evaluate the use of selected botanicals for the control of this pest.

MATERIALS AND METHODS

Experimental Site

All experiments were conducted at the Crop Research Garden and Entomology laboratory of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Ibadan, Nigeria (27±2°C, 70±5% and 12:12 hour photoperiod).

Insect Culture

Pawpaw fruits infested with *P. marginatus* were collected from the Biological Control Unit of National Horticultural Research Institute (NIHORT), Idi-Ishin, Jericho, Ibadan. They were kept at the Entomology research laboratory of the Department of CPEB where a larger culture was raised using pawpaw seedlings (8-12 weeks old).

Source of planting materials

Pawpaw seedlings of about 12 weeks were obtained from National Horticultural Research Institute (NIHORT), Idi-Ishin, Jericho, Ibadan.

Collection of botanicals

The botanicals (*Hyptis suaveolens*, *Eugenia aromatica* and *Azadirachta indica*) used for this experiment were all sourced locally within the premises of the University of Ibadan, Ibadan, Nigeria. Table 1 shows the source and description of the botanicals. The botanicals collected were washed with sterile distilled water until the dirt was completely removed and were shade dried, after which they were finely ground using Scanfrost Electric Blender (Model: SFKAB410) to obtain smaller particles of about 500 µm.

Table 1: Source and Description of Botanicals Evaluated as Protectants

Botanical	Family	Common name	Part used	Source
<i>Eugenia aromatica</i>	Myrtaceae	Clove	Fruit	Bode, Ib.
<i>Hyptis suaveolens</i>	Lamiaceae	Chan/Pignut	Leaves	Ajibode, Ib
<i>Azadirachta indica</i>	Meliaceae	Neem	Fruit	Within UI

Bode Ib = Bode market, Ibadan, Ajibode Ib = Ajibode area, Ibadan, UI= University of Ibadan

Preparation of ethanolic and aqueous extracts of *Hyptis suaveolens*, *Eugenia aromatica* and *Azadirachta indica*

Ethanolic extraction was carried out using the method described by Lalone *et al.* (2007) and this involved using 500g of ground shade dried *H. suaveolens* leaves, *E. aromatica* fruits and *A. indica* fruits which were transferred into the soxhlet extractor using analytical grade ethanol (99%) as the solvent. The ethanol was removed by evaporating the extract in a rotary evaporator at 40-60°C until a semi-liquid oil extract was obtained. The oil extracts was further concentrated in a rotary evaporator under reduced pressure. The semi-liquid oil extracts was scooped into collection tubes and kept in a refrigerator for subsequent use.

Aqueous extraction was carried out following the method described by Okunlola

et al. (2008), and this involved adding 200g of ground shade dried *H. suaveolens*, *E. aromatica* and *A. indica* seeds into 1 litre of distilled water and the mixtures were allowed to stand for 24 hours after which they were filtered using a cloth filter to obtain a homogenous substance that was sprayed.

Preliminary bioassay of oil extracts

Preliminary bioassay was conducted in the Entomological Research Laboratory to determine the most effective concentration at which the oil extracts of the botanicals will be formulated for application. Different concentrations of the extracts; 3%, 5% and 10% were made. These concentrations; each added to 500ml of water, were sprayed using a hand sprayer after 2 days on 10 adult mealybugs carefully placed with a camel hair brush on 10 weeks old pawpaw seedlings.

The setup was replicated four times. Effective concentration was determined using Probit analysis. Data on mortality of the mealybugs at 6hrs, 24hrs, 48hrs and 72hrs for each concentration was recorded. All data collected were subjected to arc-sine transformation to render them amenable to analysis of variance (ANOVA) at $\alpha.05$. After the preliminary experiment, the oil extracts were then formulated thus; *A. indica* 5%, *H. suaveolens* 10% and *E. aromatica* 10% for the bioassay.

Bioassay of ethanolic and aqueous extracts of *Hyptis suaveolens*, *Eugenia aromatica* and *Azadirachta indica* against *P. marginatus*

Twelve (12) screen-cages (1.80x0.60x1.50m) each with 15 *C. papaya* seedlings were set up at the Crop Garden of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan. Ten (10) adult mealybugs were carefully placed on each

plant using a fine camel hair brush and spraying of plants with the extracts commenced two days later. Each screen-cage served as a separate experimental unit and received a different treatment. The plant extracts were sprayed daily for three days. Synthetic insecticide (Cypermethrin®) at recommended dosage of 100 ml a.i ha⁻¹ was sprayed to serve as check. Mortality was corrected for using Schneider-Orelli's (1947) formula. All data collected were arc-sine transformed before they were analyzed using analysis of variance (ANOVA) at $\alpha.05$.

Phytochemical Analysis of Plant Extracts

Phytochemical screening for secondary compounds was performed using various methods to validate the presence and quantify the Phytoconstituents (Table 2). The botanicals were screened for saponins, alkaloids, terpenoids, phenols, flavonoids and cardinolides.

Table 2: Methods of Determination of Secondary Metabolites

Secondary metabolite	Method of determination	Remark
Saponins	Frothing	Devmurari, 2010
Alkaloids	Mayer	Chandrashekar <i>et al.</i> , 2010
Terpenenoids	Vanillin-H ₂ SO ₄	Doneva Šapceska <i>et al.</i> , 2006
Phenols	Folin–Ciocalteu	Chan <i>et al.</i> , 2007
Flavonoids	Sodium hydroxide	Ayoola, <i>et al.</i> ,2008
Cardinolides	Kedde	Manpreet, 2015

RESULTS

Preliminary Bioassay of Ethanolic Plant Extracts of *E. aromatica*, *H. suaveolens* and *A. indica* against Adult *P. marginatus*

The effectiveness of the different plant extracts evaluated for contact toxicity against *P. marginatus* in the laboratory varied significantly with the different botanicals, doses and period of exposure (Table 3). Adult mortality of *P. marginatus* varied with

period of exposure, ranging from 6.14-33.00% at 6hours to 35.21-90.00% after 72 hrs of exposure to the plant extracts.

The 10% concentration of *A. indica* had 33% mortality after 6hrs of exposure, 45% at 24 hrs, 61% after 48hrs and 90% after 72 hrs but this didn't differ significantly ($P>0.05$) from the 5% concentration which had 28.78% after 6 hrs, 39.15% after 24 hrs, 70.07% at 48 hrs and 84.15% at 72 hrs of

exposure. Both the 5% and the 10% concentrations differed significantly ($P<0.05$) from the 3% concentration which had the lowest mortality record with 12.28% after 6hrs, 21.15% after 24 hrs, 26.07% at 48 hrs and 35.21% at 72 hrs.

10% *E. aromatica* concentration had 28.78% mortality at 6hrs, 41.15% at 24hrs, 52.77% at 48hrs and 87.71% after 72hrs of exposure. This differed significantly ($P<0.05$) from the 5% concentration which had 21.15% mortality at 6hrs, 23.86% at 24hrs, 31.00% at 48hrs and 45.00% after 72hrs. The least mortality was recorded on the 3% concentration which had 6.14% at

6hrs, 18.43% at 24 hrs, 23.85% at 48hrs and 39.14% after 72 hrs.

Lastly, *H. suaveolens* 10% concentration recorded 26.56% mortality after 6hrs, 41.07% at 24 hrs, 52.77% at 48 hrs and 83.86% after 72 hrs of exposure. This figures differed significantly ($P<0.05$) from the 5% concentration which had 15% after 6hrs, 33% after 24hrs, 41.07% after 48 hrs and 57.70% after 72 hours. The lowest mortality was recorded on the 3% concentration which was significantly lower than the 10% concentration across all time durations with 6.14% after 6 hrs, 21.15% at 24hrs, 33% after 48hrs and finally 39.15% after 72 hrs of exposure.

Table 3: Percentage Mortality Values for Three Concentrations (3, 5 and 10%) of Ethanol Extracts of Three Plants Obtained in a Preliminary Bioassay on Adult *P. marginatus* (Mean $\pm$ SE)

Treatment	Concentration (%)	Mortality (%)			
		6hrs	24hrs	48hrs	72hrs
<i>Azadirachta indica</i>	3	12.28 $\pm$ 0.8cd	21.15 $\pm$ 3.3c	26.07 $\pm$ 5.7cd	35.21 $\pm$ 3.3cd
	5	28.78 $\pm$ 3.3ab	39.15 $\pm$ 5.8ab	70.07 $\pm$ 8.8a	84.15 $\pm$ 6.7a
	10	33.00 $\pm$ 5.8a	45.00 $\pm$ 5.8a	61.92 $\pm$ 3.3a	90.00 $\pm$ 4.0a
<i>Eugenia aromatica</i>	3	6.14 $\pm$ 0.3d	18.43 $\pm$ 0.0c	23.85 $\pm$ 3.3cd	39.14 $\pm$ 5.1c
	5	21.15 $\pm$ 3.3abc	23.86 $\pm$ 3.3c	31.00 $\pm$ 3.3cd	45.00 $\pm$ 5.8bc
	10	28.78 $\pm$ 3.3ab	41.15 $\pm$ 3.6ab	52.77 $\pm$ 3.3ab	87.71 $\pm$ 3.3a
<i>Hyptis suaveolens</i>	3	6.14 $\pm$ 0.3d	21.15 $\pm$ 3.3c	33.00 $\pm$ 5.6bcd	39.15 $\pm$ 5.7c
	5	15.00 $\pm$ 1.7bcd	33.00 $\pm$ 3.3b	41.07 $\pm$ 5.1bc	57.70 $\pm$ 4.0b
	10	26.56 $\pm$ 2.1abc	41.07 $\pm$ 3.8ab	52.77 $\pm$ 3.3ab	83.86 $\pm$ 3.3a
Control	No Treatment	0.00 $\pm$ 0.0d	0.00 $\pm$ 0.0d	5.29 $\pm$ 1.0d	7.15 $\pm$ 1.3d

Mean $\pm$ Standard error within a column followed by the same letter(s) are not significantly different at $p<0.05$ using Duncan's Multiple Range Test (DMRT)

Percentage mortality for ethanolic extracts of three botanicals on adult *P. marginatus* is presented in Figure 1. All treatments evaluated differed significantly from the control across all time intervals (6hrs-72hrs). After 72 hrs, *A. indica* recorded the highest mortality (87%). This was significantly higher than both *E. aromatica* (74%) and *H. suaveolens* (72%). There was no significant difference in the mortality

values for *E. aromatica* and *H. suaveolens* after 72hrs.

Percentage mortality of adult *P. marginatus* treated with aqueous extracts of three plants is presented in Figure 2. The aqueous extracts followed a similar trend to the ethanolic extract in that all treatments evaluated differed significantly from the control across all time intervals (6hrs-72hrs). After 72 hrs, highest mortality was recorded

on *A. indica* (54.2%) > *E. aromatica* (48.6%) > *H. suaveolens* (47.5). Although, there were no significant differences among the three extracts after 72hrs.

Figure 1: Percentage mortality of adult *P. marginatus* treated with ethanolic extracts of three plants

Figure 2: Percentage mortality of adult *P. marginatus* treated with aqueous extracts of three plants

Concentration of Secondary Metabolites in *E. aromatica*, *H. suaveolens*, and *A. indica*

The amount of the secondary metabolites: flavonoids, saponins, alkaloids, terpenoids, cardinolides and phenols varied as presented in Table 4. Phenol was the most abundant of all secondary metabolites screened for in all the plant samples. It was found to be most abundant in *A. indica* (1938.95mg/100g), followed by *H. suaveolens* (1830.30 mg/100g) then *E. aromatica* (1464.69 mg/100g). Highest concentration of flavonoids was detected in *H. suaveolens* (107.08mg/100g), followed by *E. aromatica* (94.53mg/100g) and *A. indica* had the least concentration (89.65mg/100g). Cardinolides was found to be most abundant in *H. suaveolens* (46.06mg/100g), followed by *E.*

aromatica (30.68mg/100g) then *A. indica* (23.73mg/100g). Highest concentration of saponins was detected in *A. indica* (10.33mg/100g), followed by *E. aromatica* (8.63mg/100g) then *H. suaveolens* (6.47mg/100g). Highest concentration of terpenoids was found in *H. suaveolens* (3.17mg/100g), followed by *A. indica* (2.9 mg/100g) then *E. aromatica* (2.18mg/100g). Highest percentage of alkaloids was found in *E. aromatica* (0.59%), followed by *A. indica* (0.58%) then *H. suaveolens* (0.17%).

LC₅₀ values for the three botanicals evaluated as protectants are provided in Table 5. *A. indica* which proved to be the most potent had LC₅₀=3.61 mg/kg body weight of *P. marginatus*, followed by *H. suaveolens* (LC₅₀=4.52 mg/kg) then *E. aromatica* (LC₅₀=4.64 mg/kg).

Table 4: Phytochemical constituents of *E. aromatica*, *H. suaveolens*, and *A. indica*

Metabolite composition	Plant Materials		
	<i>E. aromatica</i>	<i>H. suaveolens</i>	<i>A. indica</i>
Flavonoids (Mg/100g)	94.53	107.08	89.65
Saponins (Mg/100g)	8.63	6.47	10.33
Cardinolides (Mg/100g)	30.68	46.06	23.73
Terpenoids (Mg/100g)	2.18	3.17	2.9
Total Phenols (Mg/100g)	1464.69	1830.36	1938.48
Alkaloids (%)	0.59	0.17	0.58

Table 5: LC₅₀ Values for Three botanicals Determined Using Probit Analysis

Botanicals	LC ₅₀ (mg/kg)
<i>Azadirachta indica</i>	3.61
<i>Eugenia aromatica</i>	4.64
<i>Hyptis suaveolens</i>	4.52

DISCUSSION

Hyptis suaveolens, *E. aromatica* and *A. indica* extracts (ethanolic and aqueous) caused mortality of adult *P. marginatus*, though their effectiveness was dependent on the duration of exposure as mortality

increased with increase in duration of exposure. The preliminary results indicated that after 72hrs of exposure among all treatments, different concentration caused different mortality levels, indicating that the effectiveness of the extracts evaluated were dose dependent.

Hyptis suaveolens was able to cause mortality of adult *P. marginatus*. This is in line with the report of Musa *et al.* (2009) where *Trogoderma granarium* was successfully controlled in stored groundnut using *H. suaveolens* and the rates of

treatment were observed as an important factor affecting its toxic effect. In addition, the leaf extract of *H. suaveolens* have also been reported to reduce the population of *Spodoptera litura* and *Aphis craccivora* on groundnut (Ignacimuthu and Jayaraj, 2003). Similarly, in this study, application of *H. suaveolens* leaf extracts reduced population of *P. marginatus* with an increase in treatment rates.

Extracts of *E. aromatica* was effective as contact spray against *P. marginatus*. This agrees with the findings of Olotuah (2014) who reported the successful control of stored products insects; *Sitophilus zeamais*, *Callosobrochus maculatus*, *Sitophilus oryzae* and *Tribolium castaneum* with ethanolic extract of essential oil of *E. aromatica* and that it was also dependent on the levels of concentration of oil administered. Also, that *E. aromatica* is known to have pungent smell. Adedire and Lajide (2001); Longe (2004) also reported that *E. aromatica* has significant contact and fumigant action against *C. maculatus*.

Extracts of *A. indica* caused mortality of adult *P. marginatus*. This is in accordance with the reports of other researchers; Badshah *et al.* (2015) and Prishanthini and Vinobaba (2014) reported the successful control of the cotton mealybug *Phenacoccus solenopsis* using neem seed extract in Pakistan and Sri Lanka respectively. Saikia *et al.* (2000) reported that leaf (10 to 50%) and seed kernel (5%) extracts of neem caused significant mortality of the bean aphid *Aphis craccivora*. Kalyanasundaram and Das (1985) reported that plants in the families Meliaceae and Lamiaceae possess insecticidal activities which include ovicidal, larvicidal, antifeedant and repellence.

Reynolds, (1987) reported that essential oils and their constituents affect biochemical processes, which specifically disrupt the endocrinologic balance of insects. They may

be neurotoxic or may act as insect growth regulators, disrupting the normal process of morphogenesis

The phytochemical analysis revealed the presence of important secondary metabolites, many of which have been reported to possess insecticidal properties. Secondary metabolites interfere with certain vital components of the cellular signaling system and interfere with vital enzymes, involved in signaling in the nervous system and block metabolic pathways (Wink, 2000). Terpenoids and alkaloids are reported as candidates for insecticidal compounds that could be an effective alternative for insect pest management. Alkaloids have been known to possess insecticidal properties (Secoy and Smith, 1983). Terpenes causes disturbance in the nervous system leading to paralysis and mortality.

CONCLUSION

Results obtained from the bioassay demonstrated the good potential of these plants as insecticides. Thus, these plant products can be utilized for preparing botanical insecticides which is a good alternative to conventional synthetic insecticides as they are safe, economical and readily available. Therefore, *A. indica* at 5% concentration, *H. suaveolens* at 10% concentration and *E. aromatic* at 10% concentration are recommended for the control of *P. marginatus* and their formulation and production on a larger scale could also be considered beyond laboratory evaluation.

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